 DOI: 10.1002/masy.200900083

# Synthesis of Non-natural Xyloglucans by Polycondensation of 4,6-Dimethoxy-1,3,5-triazin-2-yl Oligosaccharide Monomers Catalyzed by Endo-β-1,4-glucanase

*Tomonari Tanaka, Masato Noguchi, Masaki Ishihara, Atsushi Kobayashi, Shin-ichiro Shoda**

**Summary:** A cellotetraose-backboned hepta-saccharide (XXXG) (a capital X represents a glucopyranose residue that is substituted with a xylopyranose through an α-1,6 glycosidic bond, and a capital G represents a non-substituted glucopyranose residue) and a nona-saccharide (XLLG) (a capital L represents a glucopyranose residue that is substituted with a galactopyranoseβ(1-2)xylopyranose through an α-1,6 glycosidic bond) have directly been converted to the corresponding 4,6-dimethoxy-1,3,5-triazin-2-yl derivatives (DMT-β-XXXG **1** and DMT-β-XLLG **2**, respectively) by the action of 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride (DMT-MM). The selective nucleophilic attack of the anomeric hydroxyl group to DMT-MM has been achieved in water without using any protection of the hydroxyl groups. The resulting activated oligosaccharide derivatives (**1** and **2**) were found to polymerize catalyzed by an endo-β-1,4-glucanase as catalyst. The polymerization took place in a complete regio- and stereo-selective manner, affording non-natural polysaccharides having a XXXG-repeating unit and a XLLG-repeating unit, respectively, in the main chain. It is extremely difficult to construct such definite repeating structures via the conventional synthetic routes including protection-deprotection procedures.

**Keywords:** DMT-β-glycoside; endo-β-1,4-glucanase; enzyme; polycondensation; xyloglucan

## Introduction

Xyloglucans are one of the major polysaccharides emerged in plant cell walls, and have a cellulose back bone partially substituted by a xylopyranosyl moiety through an α-1,6 glycosidic bond. Some xyloglucans extracted from *Tamarindus* seeds contain a galactopyranose unit β-1,2 linked to the xylopyranosyl moiety.[1–3] Much attention has been paid to artificial xyloglucans having a repeating oligosaccharide unit because these compounds would lead to a greater understanding of the mechanism of xyloglucan metabolism and the binding mechanism of xyloglucan to cellulose in growing plant cell wall.[4] Naturally occurring xyloglucans, however, do not possess a complete regularity of saccharide sequences. Therefore, development of an efficient method for synthesis of artificial xyloglucan derivatives with definite repeating structures has strongly been demanded.

Chemo-enzymatic method has become a strong tool for the preparation of polysaccharides having a repeating oligosaccharide unit in the main chain.[5] The synthesis of various polysaccharides having a repeating saccharide unit has been achieved by the polycondensation of disaccharide glycosyl monomers.[6–9] However, the preparation of these glycosyl monomers require laborious procedures including protection of the hydroxy groups,

Department of Biomolecular Engineering, Graduate School of Engineering, Tohoku University, 6-6-11-514, Aoba, Sendai, Miyagi 980-8579, Japan

E-mail: shoda@poly.che.tohoku.ac.jp

activation of the anomeric center by introducing chlorine or bromine, nucleophilic replacement by an appropriate leaving group, and the removal of the protecting groups (Figure 1, I→II→III→IV). These complicated procedures eventually lower the total yields of the glycosyl donor syntheses. In addition, in case of oligosaccharide glycosyl donors with higher molecular weights, the bond cleavage of labile glycosidic bonds occurs in the course of the synthetic process, affording a mixture of oligosaccharide glycosyl donors with different molecular weights.[10,11] These demerits of the conventional glycosyl donor synthesis have strongly hampered its applications to complex target molecules as well as industrial uses.

Recently we reported a facile method for direct activation of a free disaccharide by introducing a triazine ring to the anomeric center by using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM)[12–14] in an aqueous media (Figure 1, V).[15] In this paper, we wish to report that 4,6-dimethoxy-1,3,5-triazin-2-yl glycosides (DMT-glycosides) of XXXG and XLLG can be prepared directly from the corresponding oligosaccharides, and polymerized by the action of an endo-β-1,4-glucanase. The present reaction affords novel polysaccharides with well-defined repeating structures that are difficult to construct by the conventional methods.

## Experimental Part

### Materials

The substrate hepta-saccharide (XXXG) and nona-saccharide (XLLG) were purchased from Tokyo Kasei Co. Ltd., Tokyo, Japan. DMT-MM was synthesized from 2-chloro-4,6-dimethoxy-1,3,5-triazine and *N*-methylmorpholine according to the literature.[12] Endo-β-1,4-glucanase II from *Trichoderma reesei* (EGII) was purified from a crude enzyme, Celluclast® (Novozymes Japan), performing four types of column chromatography. Collected each fractions were assayed by the lanthanum–alizarin complexon method,[16] affording an active fraction that showed a single band on the SDS-PAGE. The amino acid analysis of the purified enzyme fraction clearly indicated that the sequence of the peptide fragment is identical with that of EGII.

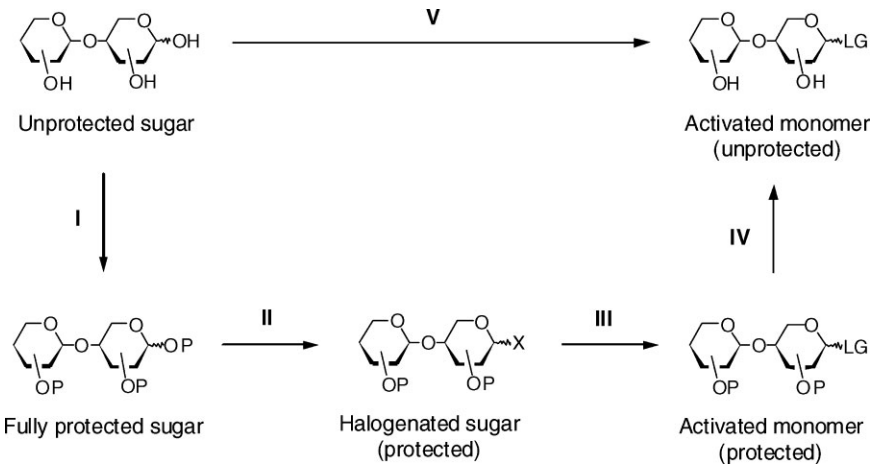



***Figure 1.***
Synthetic pathways of glycosyl donors. Step I: Protection of hydroxy group (P: protecting group), Step II: Halogenation at anomeric position (X = Cl, Br), Step III: Introduction of leaving group to anomeric position (LG: leaving group), Step IV: Deprotection, Path V: Direct anomeric activation of unprotected sugar.

### Measurements

$^1$H and $^{13}$C NMRs have been recorded on a Bruker DRX-500 at room temperature in $D_2O$. The molecular weight data were recorded by Shimadzu MALDI-TOF MS AXIMA-CFR *plus* using 2,5-dihydroxybenzonic acid as a matrix.

### Syntheses of Novel Glycosyl Monomers (Figure 2)

*4,6-Dimethoxy-1,3,5-triazin-2-yl β-xyloglucan-heptasaccharide (DMT-β-XXXG) (**1**)*

To an aqueous solution (1.00 mL) of heptasaccharide (XXXG) (53.1 mg, 50.0 μmol) and DMT-MM (27.7 mg, 100 μmol) was added 2,6-lutidine (12.0 μL, 100 μmol) and the reaction mixture was stirred for 24 h at room temperature. After confirming the complete consumption of XXXG by thin layer chromatography, the resulting adduct was purified by HPLC (column: Amide80 (21.5 × 300 mm) (TOSOH), eluent: water/acetonitrile = 2/3, flow rate: 8 mL/min, column temperature: 40 °C, detector: RI), concentrated *in vacuo*, and freeze-dried to give DMT-β-XXXG **1** (26.6 mg, 22.1 μmol, 44%).

$^1$H NMR (500 MHz, $D_2O$); δ 5.80 (1H, d, H-1 of Glc$^{I}$, $J_{1,2}$ = 8.16 Hz), 4.85-4.82 (3H, m, H-1 of Xyl$^{II–IV}$), 4.47-4.43 (3H, m, H-1 of Glc$^{II–IV}$), 3.91 (6H, s, $OCH_3$), 3.85-3.22 (39H, m, sugar-H). $^{13}$C NMR (126 MHz, $D_2O$); δ 173.3 and 171.9 (3C, triazine), 102.9 and 102.4 (3C, C-1 of Glc$^{II–IV}$), 98.8 and 98.2 (3C, C-1 of Xyl$^{II–IV}$), 96.7 (1C, C-1 of Glc$^{I}$), 79.3, 79.1, and 78.3 (3C, C-4 of Glc$^{I-III}$), 75.5, 75.4, 74.3, 74.0, 73.3, 73.0, 72.9, 72.7, 72.6, 71.8, 71.5, and 69.4 (sugar-C), 66.0 and 65.9 (3C, C-6 of Glc$^{II-IV}$), 61.5 and 61.2 (3C, C-5 of Xyl$^{II-IV}$), 59.6 (1C, C-6 of Glc$^{I}$), 55.9 (2C, $OCH_3$).

MALDI-TOF MS; calcd for $C_{44}H_{71}N_3O_{35}$ $[M + Na]^+$: 1225.0, found: 1224.6.

*4,6-Dimethoxy-1,3,5-triazin-2-yl β-xyloglucan-nonasaccharide (DMT-β-XLLG) (**2**)*

To an aqueous solution (0.2 mL) of nonasaccharide (XLLG) (27.7 mg, 20.0 μmol) and DMT-MM (11.1 mg, 40 μmol) was added 2,6-lutidine (4.6 μL, 40.0 μmol) and the reaction mixture was stirred for 24 h at room temperature. After confirming the complete consumption of XLLG by thin layer chromatography, the resulting adduct was purified by HPLC (column: Amide80 (21.5 × 300 mm) (TOSOH), eluent: water/acetonitrile = 2/3, flow rate: 8 mL/min, column temperature: 40 °C, detector: RI), concentrated *in vacuo*, and freeze-dried to give DMT-β-XLLG **2** (11.1 mg, 7.3 μmol, 36%).

$^1$H NMR (500 MHz, $D_2O$); δ 5.81 (1H, d, H-1 of Glc$^{I}$, $J_{1,2}$ = 8.05 Hz), 5.07 (2H, d, H-1 of Xyl$^{II,III}$, $J_{1,2}$ = 3.08 Hz), 4.83 (1H, d, H-1 of Xyl$^{IV}$, $J_{1,2}$ = 3.31 Hz), 4.49-4.42 (5H, m, H-1 of Glc$^{II–IV}$ and Gal$^{II,III}$), 3.92 (6H, s, $OCH_3$), 3.88-3.21 (51H, m, sugar-H). $^{13}$C NMR (126 MHz, $D_2O$); δ 173.3 and 171.9 (3C, triazine), 104.5 (2C, C-1 of Gal$^{II,III}$), 103.0, 102.6 and 102.4 (3C, C-1 of Glc$^{II–IV}$), 98.7 and 98.2 (3C, C-1 of Xyl$^{II–IV}$), 96.7 (1C, C-1 of Glc$^{I}$), 80.2 and 79.8 (3C, C-4 of Glc$^{I–III}$), 78.3, 75.5, 75.1, 74.3, 74.1, 73.5, 73.0, 72.7, 72.6, 71.9, 71.8, 71.5, 71.0, 69.4, 69.3, and 68.6 (sugar-C), 66.7, 66.6, and 65.8 (3C, C-6 of Glc$^{II–IV}$), 61.2 and 61.0 (5C, C-5 of Xyl$^{II–IV}$ and C-6 of Gal$^{II,III}$), 59.6 (1C, C-6 of Glc$^{I}$), 55.9 (2C, $OCH_3$).

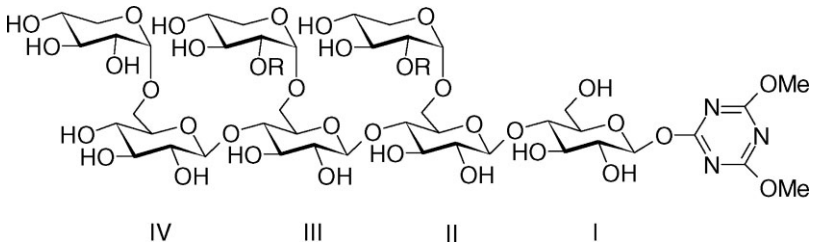



***Figure 2.***
DMT-β-XXXG **1** (R = H) and DMT-β-XLLG **2** (R = β-D-galactopyranosyl (Gal)).

MALDI-TOF MS; calcd for $C_{56}H_{91}N_3O_{45}$ $[M+Na]^+$: 1549.3, found: 1551.5.

### Enzymatic Polycondensation of 1

A mixture of DMT-β-XXXG **1** (12.0 mg, 10.0 μmol) and EGII (200 μL, 1.99 mg) in 50 mM sodium acetate buffer pH 5.0 (300 μL) was incubated at 30 °C for 4 days. After inactivation of EGII (90 °C for 10 min), the polycondensation products were isolated by HPLC (column; Amide80 (4.6 × 250 mm) (TOSOH), elute; water/acetonitrile = 1/1, flow rate; 1 mL/min, column temperature; 40 °C, detector; RI), concentrated *in vacuo*, and freeze-dried. The products $(XXXG)_2$ (1.52 mg, 0.72 μmol, 14%), $(XXXG)_3$ (0.59 mg, 0.19 μmol, 6%), and $(XXXG)_4$ (0.22 mg, 0.052 μmol, 2%) were obtained as white solids. The hydrolyzed compound of **1**, XXXG, was obtained (4.25 mg, 4.00 μmol, 40%).

**$(XXXG)_2$** (Figure 3, R = H): $^1$H NMR (500 MHz, $D_2O$); δ 5.10 (0.4H, d, H-1α of $Glc^I$, $J_{1,2}$ = 3.69 Hz), 4.84 (6H, m, H-1 of $Xyl^{II–IV,VI–VIII}$), 4.54 (0.6H, d, H-1β of $Glc^I$, $J_{1,2}$ = 8.32 Hz), 4.44 (7H, m, H-1 of $Glc^{II–VIII}$), 3.90-3.20 (78H, m, sugar-H). $^{13}$C NMR (126 MHz, $D_2O$); δ 102.9 and 102.5 (C-1 of $Glc^{II–VIII}$), 98.8 and 98.2 (C-1 of $Xyl^{II–IV,VI–VIII}$), 95.7 (C-1β of $Glc^I$), 91.9 (C-1α of $Glc^I$), 79.3, 79.1, 78.8 and 78.7 (C-4 of $Glc^{I–VII}$), 75.5, 74.7, 74.6, 74.3, 74.0, 73.8, 73.4, 73.3, 73.0, 72.7, 72.6, 71.4, 71.1, 69.9, 69.4 (sugar-C), 66.0 and 65.9 (C-6 of $Glc^{II–IV,VI–VIII}$), 61.5 and 61.2 (C-5 of $Xyl^{II–IV,VI–VIII}$), 60.0 and 59.8 (C-6 of $Glc^I$α,β).

MALDI-TOF MS; calcd for $C_{78}H_{130}O_{65}$ $[M+Na]^+$: 2130.8, found 2130.8.

**$(XXXG)_3$:** MALDI-TOF MS; calcd for $C_{117}H_{194}O_{97}$ $[M+Na]^+$: 3175.7, found 3174.2.

**$(XXXG)_4$:** MALDI-TOF MS; calcd for $C_{156}H_{258}O_{129}$ $[M+Na]^+$: 4220.6, found: 4218.1.

### *Enzymatic polycondensation of 2*

A mixture of DMT-β-XLLG **2** (4.6 mg, 3.0 μmol), and EGII (60 μl, 0.60 mg) in 50 mM sodium acetate buffer (pH 5.0) (90 μl) was incubated at 30 °C for 4 days. After inactivation of EGII (90 °C for 10 min), the polycondensation products were isolated by HPLC (column; Amide80 (4.6 × 250 mm) (TOSOH), elute; water/acetonitrile = 1/1, flow rate; 1 mL/min, column temperature; 40 °C, detector; RI), concentrated *in vacuo,* and freeze-dried. The products $(XLLG)_2$ (0.36 mg, 0.13 μmol, 9%) and $(XLLG)_3$ (0.18 mg, 0.044 μmol, 4%) were obtained as white solids. The hydrolyzed compound of **2**, XLLG, was obtained (1.87 mg, 1.35 μmol, 45%).

**$(XLLG)_2$** (Figure 3, R = β-Gal)**:** $^1$H NMR (500 MHz, $D_2O$); δ 5.11 (0.4H, d, H-1α of $Glc^I$, $J_{1,2}$ = 3.49 Hz), 5.06 (4H, m, H-1 of $Xyl^{II,III,VI,VII}$), 4.83 (2H, d, H-1 of $Xyl^{IV,VIII}$, $J_{1,2}$ = 3.43 Hz), 4.55 (0.6H, d, H-1β of $Glc^I$, $J_{1,2}$ = 7.86 Hz), 4.43 (11H, m, H-1 of $Glc^{II-VIII}$ and $Gal^{II,III,VI,VII}$), 3.90-3.20 (102H, m, sugar-H). $^{13}$C NMR (126 MHz, $D_2O$); δ 104.5 and 104.4 (C-1 of $Gal^{II,III,VI,VII}$), 103.0, 102.5, and 102.3 (C-1 of $Glc^{II-VIII}$), 98.7 and 98.2 (C-1 of $Xyl^{II-IV,VI-VIII}$), 95.8 (C-1β of $Glc^I$), 80.2, 80.1, and 79.7 (C-4 of $Glc^{I-VII}$), 75.5, 75.1, 74.0, 73.9, 73.6, 73.4, 73.0, 72.7, 72.6, 71.8, 71.5, 71.0, 70.0, 69.4, 69.3, and 68.5 (sugar-C), 66.6 and 65.8 (C-6 of $Glc^{II-IV,VI-VIII}$), 61.5, 61.1, and 61.0 (C-5 of $Xyl^{II-IV,VI-VIII}$, C-6 of $Gal^{II,III,VI,VII}$), 60.0 and 59.9 (C-6 of $Glc^I$α,β).

MALDI-TOF MS; calcd for $C_{102}H_{170}O_{85}$ $[M+Na]^+$: 2779.4, found: 2779.6.

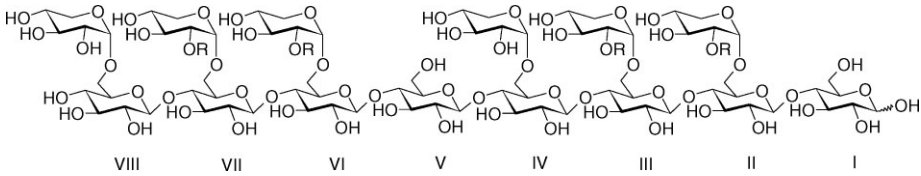



***Figure 3.***
Structure of oligoxyloglucan dimer (R = H or β-Gal).

**(XLLG)$_3$:** MALDI-TOF MS; calcd for $C_{153}H_{254}O_{127}$ $[M + Na]^+$: 4148.6, found: 4148.3.

## Results and Discussion

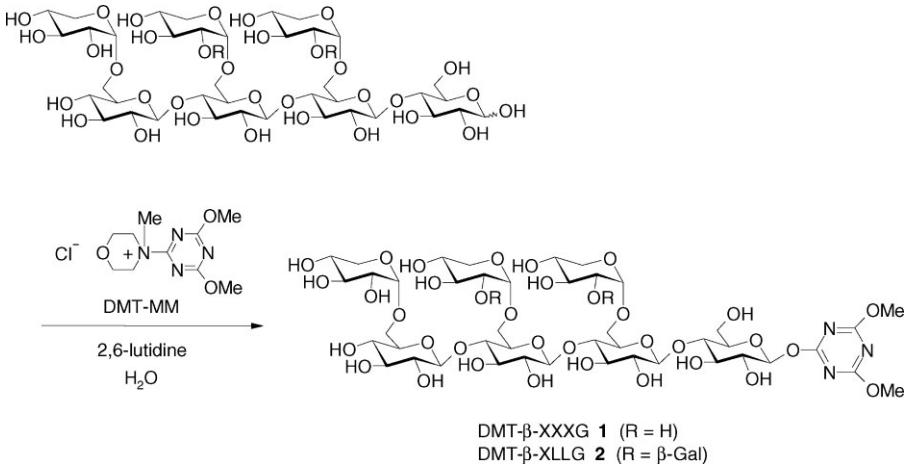
### Syntheses of Glycosyl Monomers

When an aqueous solution of hepta-saccharide (XXXG) or nona-saccharide (XLLG) was treated with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl morpholinium chloride (DMT-MM) in the presence of 2,6-lutidine, a novel glycosyl adduct **1** (DMT-β-XXXG) or **2** (DMT-β-XLLG) was formed (Scheme 1). The NMR spectrum the reaction mixture showed that the XXXG and XLLG were completely consumed, and converted to the corresponding adducts, **1** and **2**, respectively. These results indicate that the anomeric hemiacetal hydroxy groups attacks to the 2 position of the triazine ring in DMT-MM under mild conditions starting from the corresponding unprotected oligosaccharides without affecting the β-1,4 and α-1,6 glycosidic bonds of the starting oligosaccharides.

There are three kinds of hydroxy groups in the XXXG or XLLG unit, one hemiacetal hydroxy group, the primary hydroxy groups, and secondary hydroxy groups, showing their specific p$K_a$ values, 12.2, 16.0, and 16.5, respectively. On the other hand, water has the p$K_a$ value (15.7) in between those of the hemiacetal and the other hydroxy groups. The preferential attack of the hemiacetal hydroxy group to DMT-MM in water can be explained by the fact that the hemiacetal hydroxy group behaves as a stronger nucleophile than other hydroxy groups as a result of selective deprotonation by 2,6-lutidine as general base in water.

The $^1$H NMR spectrum of **1** showed a doublet peak at 5.80 ppm derived from the anomeric proton with the coupling constant of about 8.16 Hz, clearly indicating that the anomeric configuration of the adduct is β-type (Figure 4). The other anomeric protons of xylose (Xyl) and glucose (Glc) were detected at 4.8 and 4.4 ppm, respectively. The $^{13}$C NMR spectrum showed signals due to the C-4 of the Glc unit at around 79 ppm, and the C-6 of Glc-Xyl unit at 66 ppm. The structure of nona-saccharide adduct **2** was also confirmed by $^1$H and $^{13}$C NMR spectra.

In these reactions, disubstitued α-adducts having a DMT moieties at the 1 and 2 position of the oligosaccharides were formed as by-products. However, these compounds could easily been removed by a high performance liquid chromatography




***Scheme 1.***
Direct synthesis of DMT-β-XXXG **1** (R = H) and DMT-β-XLLG **2** (R = β-Gal).

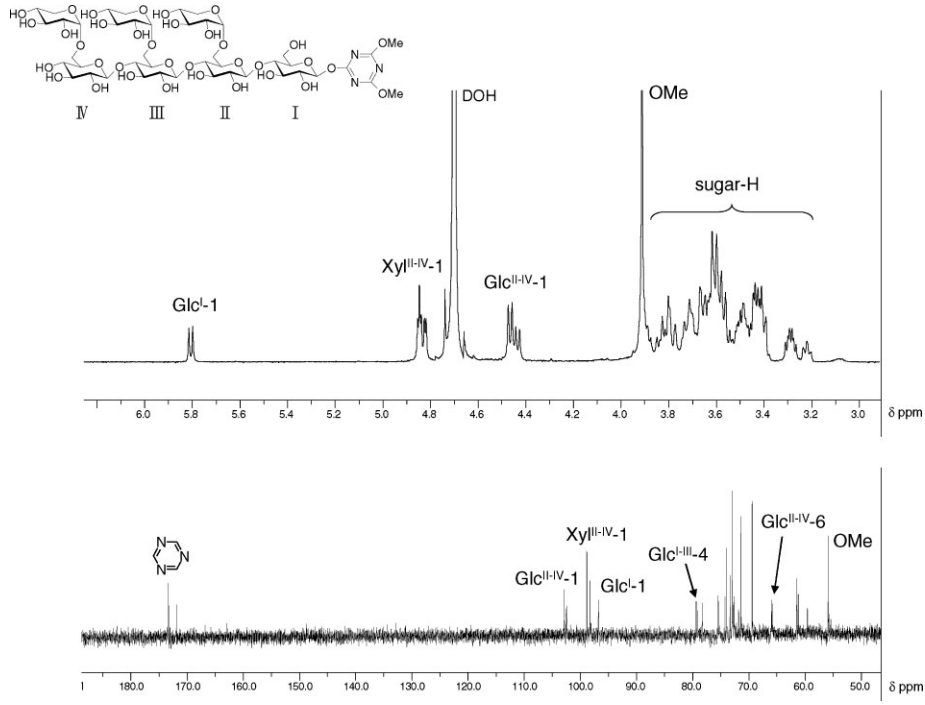
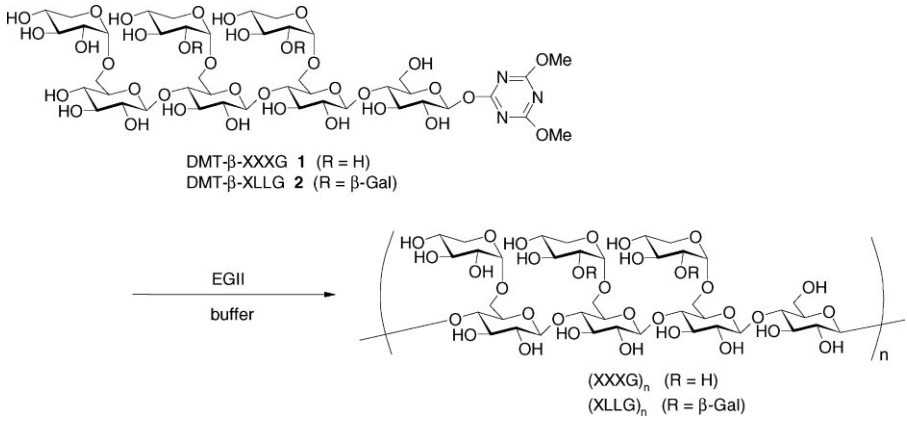



***Figure 4.***
$^{1}$H and $^{13}$C NMR spectra of DMT-β-XXXG **1**.

(HPLC), because there are large difference of physical properties between the mono-substituted adduct and the disubstituted by-product.

**Enzymatic Polycondensation Reactions**

When a solution containing a catalytic amount (13-16 wt.% for **1** or **2)** of endo-β-1,4-glucanase II from *Trichoderma reesei* (EGII) was added to a solution of the new glycosyl monomers, DMT-β-XXXG **1** and DMT-β-XLLG **2**, in 50 mM sodium acetate buffer (pH 5.0) and the resulting mixture was kept at 30 °C for 4 days, the polycondensation of these monomers took place, giving rise to the corresponding




***Scheme 2.***
Synthesis of artificial xyloglucans using DMT-β-glycosides as glycosyl monomers catalyzed by EGII.

polycondensation products having an XXXG repeating unit and an XLLG repeating unit, respectively (Scheme 2). Endo-β-1,4-glucanase III from *Trichoderma reesei* (EGIII) and a xyloglucan-specific endo-β-1,4-glucanase from *Aspergillus aculeatus* [17] also promoted the polycondensation of these glycosyl monomers.

The MALDI-TOF MS spectra of the reaction mixtures after 4 days showed the

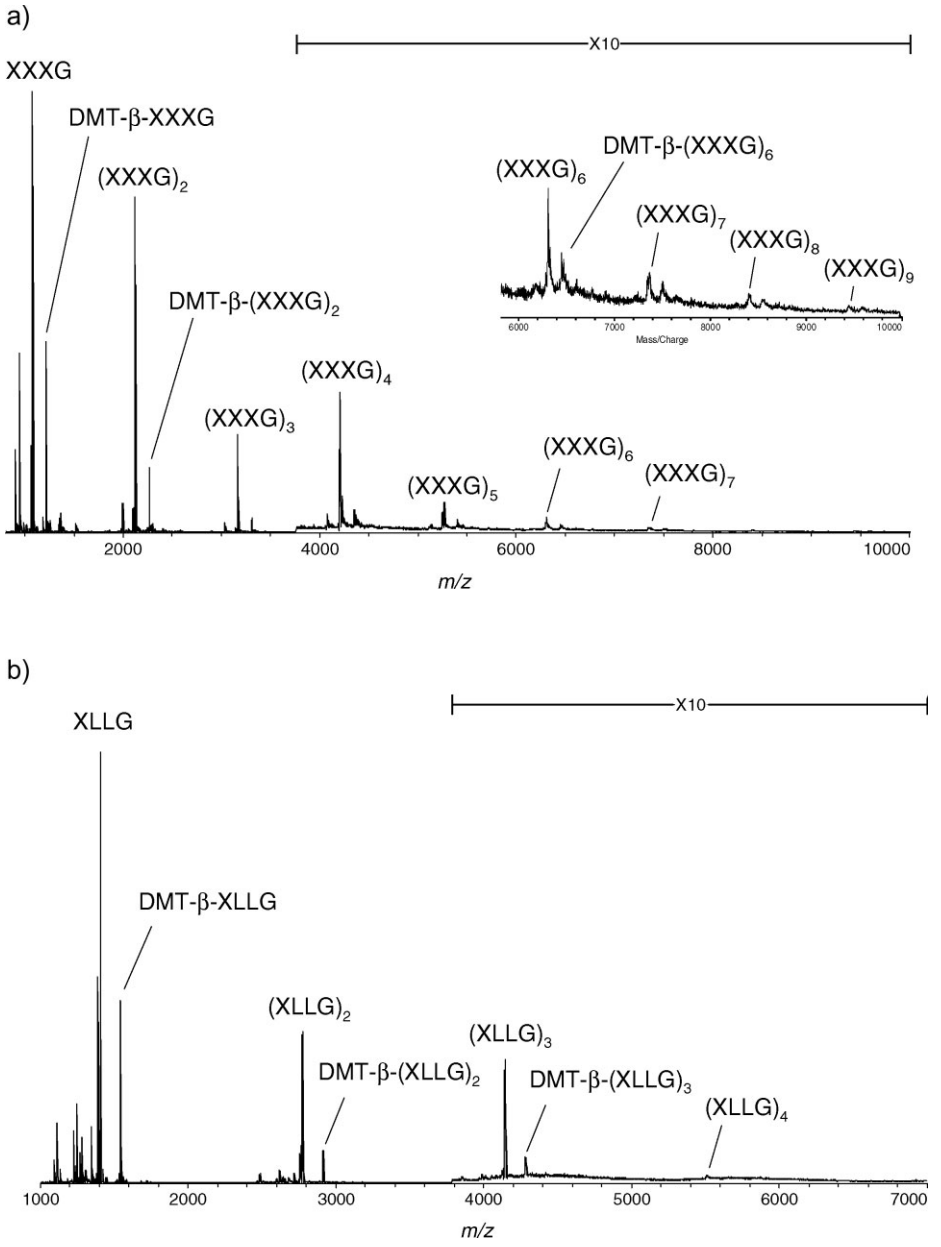



***Figure 5.***
MALDI-TOF MS spectra of the reaction mixture of EGII-catalyzed polycondensation of (a) DMT-β-XXXG **1** and (b) DMT-β-XLLG **2** as glycosyl monomers.

signals derived from the polycondensation products ($(XXXG)_n$ and $(XLLG)_n$) (Figure 5(a) and 5(b), respectively). The molecular weight differences between each peak were 1044 and 1368, respectively, which correspond to the molecular weights of the XXXG unit and XLLG unit. These results clearly indicate that the oligosaccharide transfers took place at the position of DMT-glycosidic bonds in the monomers **1** and **2**; no transglycosylations occurred at the inner positions of the XXXG or XLLG unit.

In order to increase the yields, we examined the transglycosylations under various reaction conditions. Figure 6 shows the pH effect on the polymerization of DMT-β-XXXG **1** in 40 mM citric buffer (pH = 3.0–6.0) at 30 °C, which was monitored by MALDI-TOF-MS. When the reaction was carried out at pH 5.0, the products up to hexamer ($(XXXG)_6$) were detected. Further change of pH to 6.0 caused the decrease of degree of polymerization to tetramer. Under more acidic conditions (pH = 3.0, 4.0), the polycondensation products could not be obtained. It has already been reported that the optimum pH of EGII is in the range of 3.0 to 4.0. It is assumed that the resulting polycondensation products were hydrolyzed by the action of EGII at pH 3.0 or 4.0. These results indicates that the optimum pH for transglycosylation is slightly higher than that for hydrolysis of glycosidic bonds.

When the reaction was carried out at a higher temperature (37 °C), the yield of polycondensation decreased probably because the resulting products were hydrolyzed immediately. At a lower temperature (25 °C), the polycondensation did not occur due to the inactivation of the enzyme catalyst. It is well known that addition of a water-miscible organic solvent like acetonitrile enhances the transglycosylation reaction in enzymatic glycosylations catalyzed by glycosidases. However, when the reaction was carried out in the presence of acetonitrile, the yield decreased even at the optimum conditions (pH 5.0, 30 °C) probably due to the inactivation of the enzyme.

### Isolation and Characterization of Polycondensation Products

In order to obtain more information about the structures of the enzymatic polycondensation products, the reaction mixture was chromatographed, giving rise to $(XXXG)_2$ (14% yield), $(XXXG)_3$ (6% yield), and $(XXXG)_4$ (2% yield). The $^1$H NMR spectrum of $(XXXG)_2$ showed 14 signals derived from the anomeric protons at 5.1-4.4 ppm. The anomeric protons of Glc were observed at around 4.4 ppm, strongly suggesting that the glycosidic bond between adjacent XXXG unit is β-type. The signal due to the anomeric

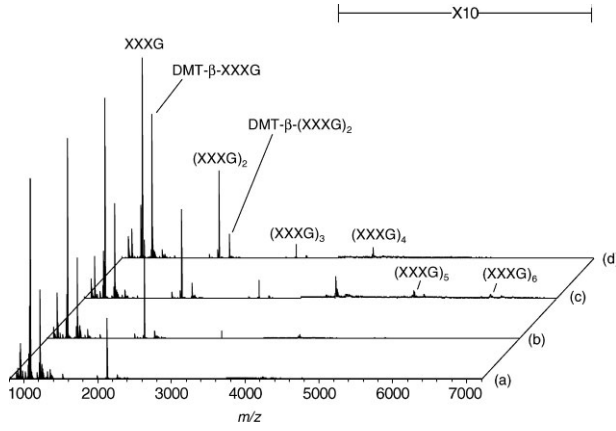



***Figure 6.***
Effect of pH on the EGII-catalyzed transglycosylation. (a) pH = 3.0, (b) pH = 4.0, (c) pH = 5.0, (d) pH = 6.0.

carbon at 102 ppm as well as the signal at 79 ppm in the $^{13}C$ NMR indicated the formation of a β-1,4 glycosidic bond. All of these data clearly showed that the polycondensation proceeded in a regio- and stereo-selective manner, affording a β-1,4 glycosidic bond. Similarly, the structure of the products, $(XLLG)_2$ (9% yield) and $(XLLG)_3$ (4% yield), has been confirmed by $^{1}H$ and $^{13}C$ NMR spectroscopy.

Xyloglucan endo-glucanase (XEG) is a glycosidase that hydrolyzes xyloglucans specifically at the reducing side of non-substituted glucose residues, i.e. the β-1,4 glycosidic bond between $Glc^{IV}$ and $Glc^{V}$ (Figure 3). The purified $(XXXG)_2$ and $(XLLG)_2$ were treated by XEG, and MALDI-TOF MS of the reaction mixture was taken. The original peaks due to $(XXXG)_2$ (Figure 7 (a-1)) and $(XLLG)_2$ (Figure 7 (b-1)) have smoothly changed to the peaks of the corresponding monomer unit XXXG (Figure 7 (a-2)) and XLLG (Figure 7 (b-2)), respectively, strongly suggesting that each unit is connected through a β-1,4 glycosidic bond.

## Reaction Mechanism

The formation of the stereoregular products can be explained by assuming the formation of a glycosyl-enzyme intermediate at an active site of EGII enzyme with the elimination of the dimethoxytriazine moiety. This reactive intermediate is then attacked by the 4''''-hydroxy group of another glycosyl monomer or propagating oligomer, which locates in the acceptor site of the catalytic site. The stereochemistry of the product is retention of configuration via double inversion with respect to the anomeric carbon atom of the glycosyl monomer. It is well known that endo-β-1,4-glucanase specifically cleaves the glycosidic bond on the non-substituted glucopyranose (G unit) of xyloglucan,[18] which is in consistent with the fact that

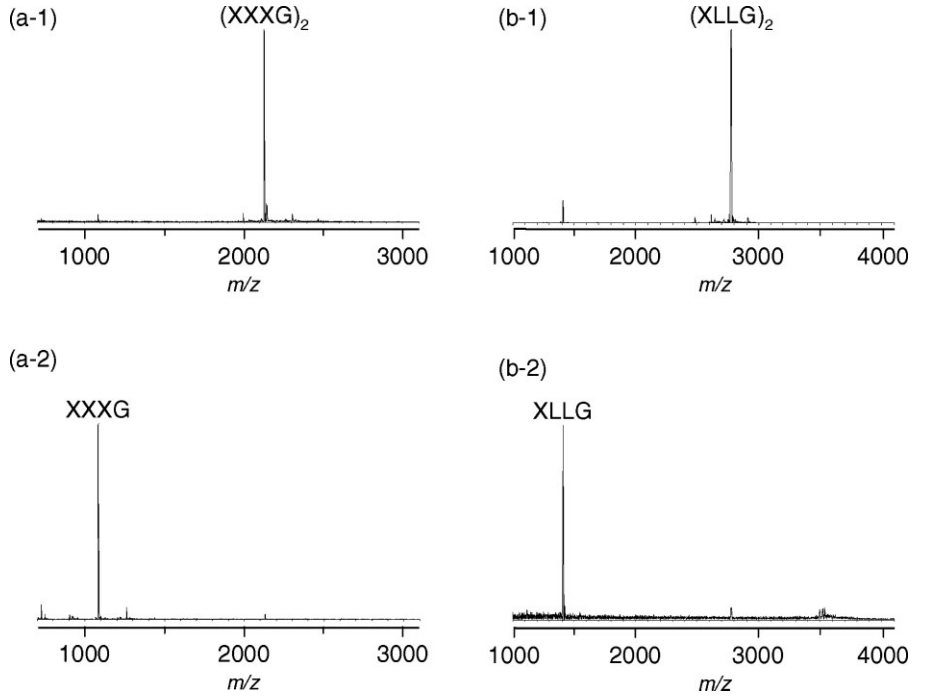



***Figure 7.***
MALDI-TOF-MS of (a-1) $(XXXG)_2$ (purified product), (a-2) hydrolysis mixture catalyzed by XEG, (b-1) $(XLLG)_2$ (purified product), (b-2) hydrolysis mixture catalyzed by XEG.

the polycondensation proceeds via heptasaccharide unit or nona-saccharide unit without cleaving any glycosidic bonds on the X unit and L unit.

## Conclusion

The artificial oligoxyloglucans having a definite repeating structure have successfully been prepared by an enzymatic polycondensation of DMT-β-glycoside monomers. These monomers can be synthesized directly from the corresponding unprotected oligosaccharides by using DMT-MM in aqueous solution. The present process is the first report of chemoenzymatic polycondensation reaction, where both of the chemical synthesis of the glycosyl monomer and the successive enzymatic reaction are achieved without using any protection of hydroxy groups in water. This new methodology would be an efficient and a practical tool for the construction of polysaccharides in glycotechnology.

*Acknowledgements:* This work was supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Sports, Science and Technology, Industrial Technology Research Grant Program in 2006 from NEDO of Japan, and the International Center of Research & Education for Molecular Complex Chemistry in 2007 from Tohoku University Global COE Program. We thank Prof. Takahisa Hayashi (Kyoto University) for providing xyloglucan-specific endo-$\beta$1,4-glucanase from *Aspergillus aculeatus* (XEG).

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